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Numerical Taxonomy of South Indian *Piper* L. II. Principal Component Analysis of the major taxa

P.N. Ravindran and K. Nirmal Babu
Indian Institute of Spices Research,
Post Bag No.1701, Kozhikode - 673 012, Kerala, India

Abstract

A principal component analysis study was carried out on the major *Piper* species occuring in South India, using 17 OTUs and 30 characters. Seven PCs were extracted by the analysis. The first PC consisting of leaf length, leaf breadth, leaf size index, petiole length, distance from the base to the second pair of ribs, plant type, fruit colour, fruit taste and thrips infestation. The second PC consists of spike length, peduncle length, spike orientation and fruit shape. The third PC consists of leaf length/leaf breadth index, rib number, growth habit, and distribution. The fourth PC consists of bract type. The fifth PC consists of leaf length/spike length index and spike shape. The sixth PC consists of guard cell length, guard cell breadth and leaf texture. The seventh PC consists of spike texture. The PC scores were plotted against the principal components and the nature of divergence of various *Piper* species could be deduced from these scatter plots. *P. nigrum* showed closest affinity with *P. wightii*. The results are discussed.

INTRODUCTION

Piper L. (Piperaceae) is a large genus distributed in Central America, Northern South America and Southern Asia. More than 3000 binomials have been reported under the genus (Index Kewensis, 1895 - 1970), many of which could be duplications. The genus includes black pepper (*Piper nigrum* L.), long pepper (*P.longum* L.), cubeb pepper (*P.cubeba* L.), Java long pepper (*P.chaba* L.), betle leaf (*P.betle* L.) and Kava (*P.methysticum* L.). All of them are used in traditional medicine. Black pepper is the most widely used spice in the world. The evergreen forests of Western Ghats of South India is the centre of diversity of *P.nigrum* and its close relatives. No monographic study has so far done on the South Indian taxa, and little is known about their inter- relationships.

The present study is a continuation of the earlier one on numerical taxonomy of Indian taxa of *Piper* (Ravindran et al,. 1992), Here the technique of principal component analysis is used to study their taxonomic relationships.

This paper is dedicated to the memory of Dr. V.V. Sivarajan, Professor of Botany, Calicut University and the then Editor of *Rheedea*, whose untimely departure left a vaccum in taxonomical research of the country.

MATERIALS AND METHODS

The present study utilised 17 Operational Taxonomic Units (OTUs) comprising of eleven species (Table 1.) The different characters and their states used in the analysis are given in Table 2. Observations on 30 characters were recorded using both live as well as herbarlum specimens. One hundred observations were recorded in each case except in the case of *P.silentvalleyensis* for which only limited quantity of material was available. Here spike characters were recorded from 25 samples, while other morphological characters were recorded from 50 samples. The mean values were used in the analysis. In the case of non metric characters the observations were recorded on a 1-5 scale (Sneath & Sokal, 1973).

Table 1 : *Piper* taxa (OTUS) used in the present study

OTU No.	SPECIES	
1.	*Piper attenuatum*	
2.	*P.argyrophyllum*	
3.	*P.galeatum*	
4.	*P.hymenophyllum*	
5.	*P.longum*	
6.	*P.mullesua*	
7.	*P.schmidtii*	
8.	*P.silentvalleyensis*	
9.	*P.trichostachyon*	
10.	*P.wightii*	
11.	*P.nigrum*	(1) Acc.No.2077
12.	*P.nigrum*	(2) Acc.No.2071
13.	*P.nigrum*	(3) Acc.No.2009
14.	*P.nigrum*	(4) Acc.No.2059
15.	*P.nigrum*	(5) Acc.No.2060
16.	*P.nigrum*	(6) Acc.No.2015
17.	*P.nigrum*	(7) Acc.No.2062

The analysis was carried out as outlined by Frane and Hill (1976) and Frane, Jenrich and Sampson (1981) at the Computer centre of the Carnegle - Mellon University, Pittsburgh, USA, using the BMDP-81 computer programme originally developed by the

Department of Biomathematics, University of California. The analysis involved the following steps: (i) The computation of the correlation matrix for the thirty characters, (ii) computation of the eigen vectors and eigen roots and the estimation of PC loadings. These indicate how far each character is correlated with the principal components, (iii) Rotation of PC loadings to obtain a simple interpretation so that each PC can be taken to be representative of a few sets of highly correlated characters, and (iv) the computation of PC scores. The PC score is a numerical value which expresses the degree to which each case or OTU possess the property that the PC describes.

Table 2. Characters and their states used in the study of *Piper* spp.

Character Code number	Details of Characters
1.	Leaf length in mm
2.	Leaf breadth in mm
3.	Leaf length / Leaf breadth
4.	Leaf size index
5.	Petiole length in mm
6.	Spike length in mm
7.	Peduncle length in mm
8.	Leaf length /Spike length
9.	Stomatal density per mm^2
10.	Guard cell length in mm
11.	Guard cell breadth in mm
12.	Distance from leaf base to the 2nd pair of ribs
13.	Number of ribs
14.	Leaf shape (1: ovate to ovate - elliptic; 2: cordate 3: ovatelanceolate; 4 : elliptictoelliptic - lanceolate)
15.	Leaf base (1:round; 2:cordate; 3:acute to attenuate)
16.	Leaf texture (1: Glabrous; 2: Sparsely hairy mainly on the veins; 3: hirsute)
17.	Leaf nature (1:membraneous; 2: coriaceous)
18.	Spike shape (1: filiform; 2: cylindrical; 3: globose)
19.	Spike orientation (1:pendulous; 2:erect)

20.	Spike texture (1:glabrous; 2:hirtellous)
21.	Bract type (1: sessile, adnate to rachis; 2: stalked, peltate, or bicular; 3:cupular with decurrentbase; 4:fleshy, connate,cup-like; 5:oblong, angular and free all around)
22.	Stamen number (1:two; 2: three or four)
23.	Fruit nature (1: free; 2: fused)
24.	Fruit shape (1: ovate-oblong; 2:spherical; 3:ellipti cal; 4:obovate)
25.	Fruit colour change on ripening (1:green to orange and red: 2: green to yellow; 3:green to black)
26.	Fruit taste (1: pungent; 2: spicy and mildly pungent; 3:bitter)
27.	Plant type (1: dioecious; 2: monoecious; 3: predominantly monoecious)
28.	Growth habit (1: shrubby climber; 2: stout woody climber; 3: no climbing habit and trailing on the ground)
29.	Distribution in the natural habitat [1:plains to lower elevations (from 0 - 500 m); 2: plains to higher elevations (from 0-1500 m) 3: lower elevations to higher elevations (from 500 - 1500 m); 4: found only at high elevations (above 1500 m)].
30.	Presence of Thrips infestation (1: present; 2:absent).

RESULTS AND DISCUSSION

Table 3 gives the PC of 30 characters with the variance explained by each PC, and the cumulative proportion of variance explained by successive PC. It is seen from this that the cumulative proportion of variance explained by the PCs increase rapidly for the first four PCs and then slowly upto the seventh, beyond which the increase is negligible, indicating that the first seven PCs alone are important in contributing to the variability observed among the OTUs. The first four PCs together account for 78% of the variability, and the first seven PCs together accounted for 92% of the total variations explained by the 30 characters.

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Table 3. Variance explained by the Principal Components and the cumulative proportion of variance

FACTOR	VARIANCE EXPLAINED	CUMULATIVE IN DATA SPACE	PROPORTION OF VARIANCE IN FACTOR SPACE
1.	10.2517	0.3417	0.3706
2.	6.6616	0.5638	0.6115
3.	3.8556	0.6923	0.7509
4.	2.9259	0.7898	0.8566
5.	1.4423	0.8379	0.9088
6.	1.3628	0.8833	0.9581
7.	1.1599	0.9220	1.0000
8.	0.7804	0.9480	
9.	0.4869	0.9642	
10.	0.3568	0.9761	
11.	0.3093	0.9864	
12.	0.1594	0.9918	
13.	0.1166	0.9956	
14.	0.0636	0.9978	
15.	0.0398	0.9991	
16.	0.0273	1.0000	
17.	0.0000	1.0000	
18.	0.0000	1.0000	
19.	0.0000	1.0000	
20.	0.0000	1.0000	
21.	0.0000	1.0000	
22.	0.0000	1.0000	

Table 4 gives the loadings for the seven PCs after orthogonal rotation and arranging the columns and rows, so that the columns appear in decreasing order of variance explained by the PC; and the rows arranged so that loadings greater than 0.5 appear first. The first PC is having high loadings for the characters leaf length, leaf breadth, leaf size index, petiole length, distance from the base to the second pair of ribs, plant type, fruit colour, fruit taste, and thrips infestation. Thus the first PC represents these characters. Characters such as spike length, penduncle length, spike orientation and fruit shape have high loadings on the second PC. In other words the second PC consists of these characters. Similarly the third PC consists of leaf length/leaf-breadth ratio, rib number, growth habit, and distribution. The fourth PC consists of bract type. The fifth PC consists of leaf length/spike length ratio, and spike shape. The sixth PC consists of guard cell length, guard cell breadth, and leaf texture. The seventh PC consists of spike texture.

Table 4. Sorted, rotated PC loadings

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
30	-0.947	0.000	0.000	0.000	0.000	0.000	0.000
4	0.928	-0.258	0.000	0.000	0.000	0.000	0.000
1	0.922	-0.321	0.000	0.000	0.000	0.000	0.000
5	0.906	-0.288	0.000	0.000	0.000	0.000	0.000
12	0.890	0.000	0.000	0.304	0.000	0.000	0.000
27	0.861	0.000	0.000	0.000	0.000	0.000	0.000
26	-0.860	-0.323	-0.304	0.000	0.000	0.000	0.000
2	0.850	-0.331	0.325	0.000	0.000	0.000	0.000
25	-0.823	0.000	0.000	0.287	0.350	0.000	0.000
24	0.000	0.949	0.000	0.000	0.000	0.000	0.000
7	0.000	-0.855	0.000	0.000	0.000	0.000	0.329
8	0.000	0.791	0.000	0.000	0.000	-0.299	0.000

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13	-0.450	0.773	0.000	0.000	0.000	0.000	0.000
15	0.000	0.735	0.000	0.000	0.000	0.000	0.299
14	-0.327	0.712	-0.328	0.000	0.000	0.000	0.000
22	-0.445	-0.568	-0.257	-0.466	0.000	0.000	0.000
23	-0.397	0.000	0.368	0.000	0.000	0.000	0.000
13	-0.380	0.000	0.324	0.000	0.000	0.000	-0.267
29	-0.519	0.000	-0.808	0.000	0.000	0.000	0.000
28	0.000	0.000	-0.743	0.551	0.000	0.000	0.000
3	-0.306	0.376	-0.719	0.358	0.000	0.000	0.274
21	0.000	0.000	0.000	0.912	0.000	0.000	0.000
18	-0.275	0.484	0.000	0.000	0.808	0.000	0.000
8	0.000	0.547	0.000	0.000	0.786	0.000	0.000
17	0.474	0.492	0.000	0.000	-0.650	0.000	0.000
10	0.000	0.000	-0.312	0.000	0.000	0.808	0.000
16	-0.343	-0.278	0.000	0.396	0.000	0.727	0.000
11	0.375	0.000	0.000	0.000	0.000	0.722	-0.372
20	0.000	0.000	0.000	0.000	0.000	0.000	0.914
9	0.000	0.352	0.472	0.457	0.000	0.000	-0.501

Table 5. Estimated PC scores

CASE NO.	FACTOR 1	FACTOR 2	FACTOR 3	FACTOR 4	FACTOR 5	FACTOR 6	FACTOR 7
1	-0.704	-1.056	-0.459	-1.835	-0.037	-0.348	-0.072
2	-0.396	-1.582	-0.514	-1.688	-0.383	-1.297	-0.130

3	-0.916	-1.685	-0.745	-1.024	-0.613	-0.052	0.226
4	-1.098	-0.848	-0.083	-0.758	-0.274	3.247	-0.170
5	-1.540	0.695	3.366	-0.175	-0484	-0.568	-0.054
6	-0.393	1.699	-1.225	-0.098	3.177	-0.200	-0.226
7	-1.223	-1.265	-1.560	2.238	0.288	-1.074	-1.014
8	-0.896	2.466	-1.033	-0.753	-2.097	-0.698	-0.743
9	-0.634	-0.412	-0.451	1.031	-0.431	-0.001	2.728
10	0.515	-0.047	0.444	0.477	-0.376	0.446	-0.857
11	1.252	0.216	-0.108	-0.300	-0.017	-0.407	0.304
12	0.840	-0.141	0.177	-0.047	-0.376	-0.446	-0.857
13	1.059	0.089	0.172	-0.111	-0.045	0.037	-0.071
14	0.779	0.319	0.215	0.946	-0.260	-0.804	-0.997
15	1.222	-0.225	0.229	0.424	0.093	0.451	-0.498
16	1.214	-0.050	0.369	-0.240	0.089	-0.303	-0.387
17	0.919	-0.196	0.205	-0.135	0.059	0.037	2.126

Table 5 gives the PC scores for each of the 17 OTUs. These scores give information on the extent of the relationship between the PC and the OTUs. The PC scores can be used for the construction of the dispersion maps of *Piper* species by plotting against two axes, each axis representing a PC. Figs.1-3 shows the dispersion maps of *Piper* species with regard to the first three PCs, taking two at a time. It is seen from Fig.1 that eight OTUs (10-17) are grouped into a close cluster which includes *P.wightii* (10), *P.nigrum* (11-16) and *P.nigrum* var. *hirtellosum* (17). OTUs 6 and 8 (*P.mullesua* and *P.silentvalleyensis*) are having large differences on the Y-axis, representing PC2 (Spike length, peduncle length, spike orientation, fruit shape), thereby indicating that these characters are important in differentiating these species from others. OTUs 1 and 2 (*P.attenuatum* and *P.argyrophyllum*) show close association indicating their closeness with regard to these PCs. Other OTUs 1 and 2

(*P.attenuatum* and *P.argyrophyllum*) show close association indicating their closeness with regard to these PCs. Other OTUs are well separated in the plot, thereby showing their relative independence in relation to the 1st an 2nd PCs.

In Fig.2 it can be seen that OTUs (*P.longum*) exhibits a very large difference on the Y-axis representing PC3 (leaf length/leaf breadth, rib number, growth habit, distribution), thereby indicating that this taxa gets differentiated from all other taxa based on the above set of characters. Here *P.nigrum* and *P.wightii* form one close group; while OTU6 (*P.mullesua)* and 8 *(P.silentvalleyensis)* are some what away from the Y-axis, and 4 *(P.hymenophyllum* away from the X-axis representing PC 1. The other OTUs are loosely associated as a cluster in which 1,2,3 and 9 (*P.attenuatum), P.argyrophyllum* , *P.galeatum,* and *P.trichostachyon)* respectively are more close to one another, indicating their relative closeness with regard to PC 1 and 3. The dispersion of the OTUs with regard to PCs 2 and 3 shows the distant positioning of *P.longum, P.silentvalleyensis,* and *P.mullesua.*

Similarly Fig.3 represents the distribution of OTUs between 2nd and 3rd PCs. This gives a very interesting distribution where OTUs belonging to *P.nigrum* (OTUs 11-17) and *P.wightii* form one close group around the mid point indicating that PCs 2 and 3 do not play any role in the divergence of these two species, and that these two species are very much related. As in the previous figure *P.longum* (5) occupies a unique position here also. *P.mullesua* and *P.silentvalleyensis* occupy close locations indicating their closeness. *P.trichostachyaon* appear close to the *P.nigrum* group in this scatter plot, and that PC2 and 3 are not efficient in differentiating this from the rest. Similar scatter plots can be prepared employing the various combinations of PCs and the impact of each PC in bringing about the differentiation of the various OTUs can be studied. These scatter plots are very useful in understanding the nature of divergence and characters responsible for such divergence.

The factor scores also give us some important clues with regard to the type of characters or groups of characters that led to the differentiation of the different taxa. OTUs 1 and 2 *(P. attenuatum* and *P.argyrophyllum*) get differentiated from others mainly by PCs 2 and 4, while PC 6 differentiated *P.argyrophyllum* from others. OTU 3 (*P.galeatum*) gets separated from other taxa by PC 4. OTU4 (*P.hymenophyllum*) gets differentiated by virtue of PC 1 and 6. OTU 5 *(P. longum)* gets differentiated by PC 1 and 3. *P.mullesua* (OTU 6) is distinct from all others due to PCs 2,3 and 5. PCs 1,2,3,4,6 and 7 are important in differentiating *P.schmidtii* from other species, PC4 being the most important. PCs 2,3 and 5 are important in separating *P.silentvalleyensis* (8) from other taxa. *P.trichostachyon* (9) gets delineated from other taxa by virtue of PC 4 and 7. PC 1 is important in separating *P.nigrum* from other taxa, while PC 7 separates *P.nigrum* var.*hirtellosum* from *P.nigrum* itself.

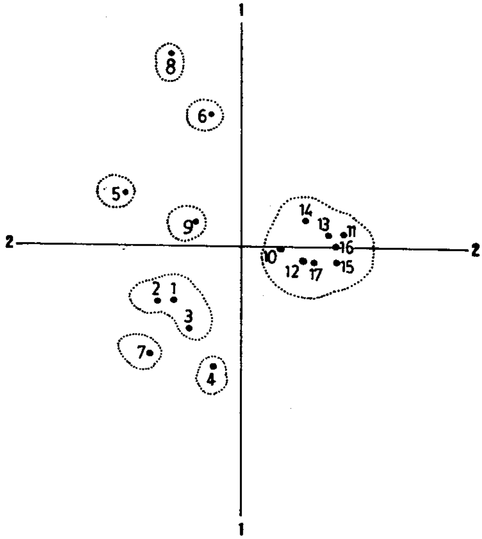

Fig.1. Scatter plot showing the distribution of OTUs between 1st and 2nd Principal Components.

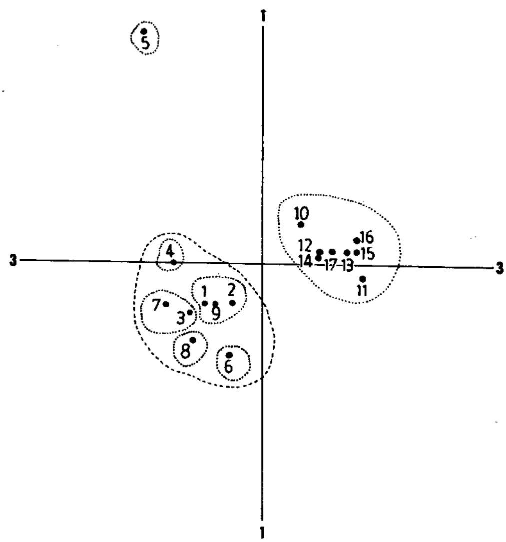

Fig.2. Scatter plot showing the distribution of OTUs between Ist and 3rd Principal Components.

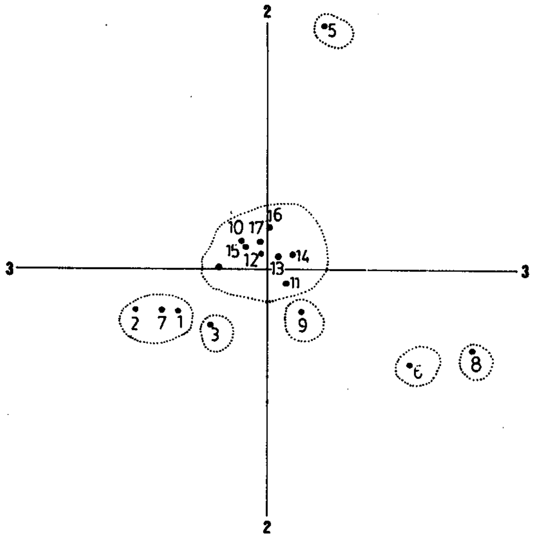

Fig. 3. Scatter plot showing the distribution of OTUs between 2nd and 3rd Principal Components.

The results of the present study also points out to certain short comings in the conventional taxonomic grouping of some of the south Indian taxa. *P.nigrum* is included under the section Eupiper along with *P.attenuatum, P.argyrophyllum, P. hymenophyllum* and *P.wightii*. This grouping is untenable as *P. nigrum* gave a distinct clustering indicating its very distinct nature, only *P.wightii* grouped with it. This result further support the results already obtained in other studies also (Ravindran et al., 1992). Infact *P.nigrum* is the only species having the alkaloid piperine and a whole set of terpenoids that contribute to the typical black pepper flavour.

P. longum showed a very distinctive grouping. This infact is a unique species in having creeping habit, erect cylindrical spikes, laterally fused flowers and fruits etc., and having a distinct anatomy (Murty, 1960). Hooker (1886) included this species in the section Chavica, along with P.mullesua, but the two differ in a many respects. *P. longum* is more closely related to *P.hapnium,* an endangered species. *P.mullesua* on the other hand showed much resemblance to *P.silentvalleyensis*. In a cluster analysis study these two were associated in a cluster (Ravindran et al.,1992), though they can be well differentiated by PCs 2 and 5.

The close association as between *P.attenuatum, P.argyrophyllum* and *P.galeatum - P.trichostachyon* were also reported by earlier studies (Rahiman & Bhagavan, 1985).

Thus the present study gives insight into the relationships among the South Indian taxa of *Piper*, especially on the characters that led to the differentiation of the individual species.

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